FILE 'MEDLINE' ENTERED AT 15:17:22 ON 17 MAR 2006

FILE 'AGRICOLA' ENTERED AT 15:17:22 ON 17 MAR 2006

FILE 'DRUGU' ENTERED AT 15:17:22 ON 17 MAR 2006
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FILE 'BIOENG' ENTERED AT 15:17:22 ON 17 MAR 2006
COPYRIGHT (C) 2006 Cambridge Scientific Abstracts (CSA)

FILE 'SCISEARCH' ENTERED AT 15:17:22 ON 17 MAR 2006
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=> s alpha amylase
L1        68460 ALPHA AMYLASE

=>

=> s l1 and rhizomucor?
L2           44 L1 AND RHIZOMUCOR?

=> s l2 not (lan?/au or wenping?/au or duan?/au or johanessen?/ au)
MISSING OPERATOR

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3            26 DUP REM L2 (18 DUPLICATES REMOVED)
                 ANSWER '1' FROM FILE MEDLINE
                 ANSWER '2' FROM FILE AGRICOLA
                 ANSWER '3' FROM FILE CABA
                 ANSWERS '4-5' FROM FILE BIOSIS
                 ANSWERS '6-20' FROM FILE CAPLUS
                 ANSWERS '21-24' FROM FILE BIOTECHDS
                 ANSWERS '25-26' FROM FILE SCISEARCH

=> d his

     (FILE 'HOME' ENTERED AT 15:17:12 ON 17 MAR 2006)

     FILE 'MEDLINE, AGRICOLA, DRUGU, JICST-EPLUS, CABA, BIOTECHNO, BIOSIS,
     CAPLUS, LIFESCI, BIOTECHDS, EMBASE, BIOENG, SCISEARCH' ENTERED AT
     15:17:22 ON 17 MAR 2006
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L1 68460 S ALPHA AMYLASE
L2 44 S L1 AND RHIZOMUCOR?
L3 26 DUP REM L2 (18 DUPLICATES REMOVED)

=> d ibib abs total l3

L3 ANSWER 1 OF 26 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 90065885 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2586234
TITLE: **Rhizomucor** miehei triglyceride lipase is processed and secreted from transformed Aspergillus oryzae.
AUTHOR: Huge-Jensen B; Andreasen F; Christensen T; Christensen M; Thim L; Boel E
CORPORATE SOURCE: Novo-Nordisk A/S, Novo Alle, Copenhagen, Denmark.
SOURCE: Lipids, (1989 Sep) Vol. 24, No. 9, pp. 781-5.
Journal code: 0060450. ISSN: 0024-4201.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199001
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900103

AB The cDNA encoding the precursor of the **Rhizomucor** miehei triglyceride lipase was inserted in an Aspergillus oryzae expression vector. In this vector the expression of the lipase cDNA is under control of the Aspergillus oryzae **alpha-amylase** gene promoter and the Aspergillus niger glucoamylase gene terminator. The recombinant plasmid was introduced into Aspergillus oryzae, and transformed colonies were selected and screened for lipase expression. Lipase-positive transformants were grown in a small fermentor, and recombinant triglyceride lipase was purified from the culture broth. The purified enzymatically active recombinant lipase (rRML) secreted from A. oryzae was shown to have the same characteristics with respect to mobility on reducing SDS-gels and amino acid composition as the native enzyme. N-terminal amino acid sequencing indicated that approximately 70% of the secreted rRML had the same N-terminal sequence as the native **Rhizomucor** miehei enzyme, whereas 30% of the secreted rRML was one amino acid residue shorter in the N-terminal. The recombinant lipase precursor, which has a 70 amino acid propeptide, is thus processed in and secreted from Aspergillus oryzae. We have hereby demonstrated the utility of this organism as a host for the production of recombinant triglyceride lipases.

L3 ANSWER 2 OF 26 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2006) on STN DUPLICATE 10
ACCESSION NUMBER: 88:5788 AGRICOLA
DOCUMENT NUMBER: IND87077883
TITLE: Improved purification of **alpha-amylase** isolated from **Rhizomucor** pusillus by affinity chromatography.
AUTHOR(S): Turchi, S.L.; Becker, T.
AVAILABILITY: DNAL (QR1.C78)
SOURCE: Current microbiology, 1987. Vol. 15, No. 4. p. 203-205
Publisher: New York, N.Y. : Springer International.
CODEN: CUMIDD; ISSN: 0343-8651
NOTE: Includes references.
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

L3 ANSWER 3 OF 26 CABA COPYRIGHT 2006 CABI on STN DUPLICATE 11
ACCESSION NUMBER: 85:105652 CABA
DOCUMENT NUMBER: 19850780723
TITLE: An enzymic method for analysis of total mixed-linkage [beta]-glucans in cereal grains
AUTHOR: Aman, P.; Hesselman, K.

CORPORATE SOURCE: Dep. of Anim. Nutr. and Management, Swedish Univ. of Agric. Sci., 750 07 Uppsala, Sweden.
SOURCE: Journal of Cereal Science, (1985) Vol. 3, No. 3, pp. 231-237. 18 ref.
ISSN: 0733-5210
DOCUMENT TYPE: Journal
LANGUAGE: English
ENTRY DATE: Entered STN: 19941101
Last Updated on STN: 19941101

AB An enzymic method for analysis of total [beta]-glucan contents of cereals was developed and its use in barley is described. The method involves complete starch degradation using a thermostable [**alpha**]-**amylase** and amylo-glucosidase, precipitation of buffer-soluble [beta]-glucans with 80% v/v ethanol and use of a [beta]-glucanase preparation from **Rhizomucor** pusillus to degrade soluble and insoluble [beta]-glucans. Buffer-soluble polymers and mono- and oligosaccharides formed from [beta]-glucans were isolated in the 80% ethanol extract and isolated sugars were hydrolysed with acid. The total mixed-linkage [beta]-glucan content was determined from the glucose content by the glucose oxidase method. The different steps and the precision of this enzymic method were assessed and results on some barley cv. using this method were compared with those of 2 previously described methods. Total [beta]-glucan contents of different barley cv., wheat, rye, triticale and oats were anlaysed by this method. All cereals contained [beta]-glucans and mean values ranged from 0.5% (w/w, DW) to 3.8%, which compared well with previous results.

L3 ANSWER 4 OF 26 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:104892 BIOSIS
DOCUMENT NUMBER: PREV200200104892
TITLE: Recombinant lipase and **alpha-amylase** variants.
AUTHOR(S): Nielsen, E. [Inventor]; Rasmussen, G. [Inventor]; Halkier, T. [Inventor]
CORPORATE SOURCE: Copenhagen, Denmark
ASSIGNEE: NOVO NORDISK A-S
PATENT INFORMATION: US 5731280 19980324
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (March 24, 1998) Vol. 1208, No. 4, pp. 3289. print.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jan 2002
Last Updated on STN: 25 Feb 2002

L3 ANSWER 5 OF 26 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 1986:311703 BIOSIS
DOCUMENT NUMBER: PREV198631035939; BR31:35939
TITLE: PURIFICATION EXTRACELLULAR **ALPHA AMYLASE** FROM **RHIZOMUCOR**-PUSILLUS.
AUTHOR(S): LANDIS D [Reprint author]; TURCHI S L; DEPLOEY J
CORPORATE SOURCE: MILLERSVILLE UNIV
SOURCE: Proceedings of the Pennsylvania Academy of Science, (1985) Vol. 59, No. 1, pp. 80.
Meeting Info.: 61ST ANNUAL MEETING OF THE PENNSYLVANIA ACADEMY OF SCIENCE, LANCASTER, PA., USA, APR. 21-23, 1985.
PROC PA ACAD SCI.
CODEN: PPASAK. ISSN: 0096-9222.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 26 Jul 1986
Last Updated on STN: 26 Jul 1986

L3 ANSWER 6 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2004:203960 CAPLUS
DOCUMENT NUMBER: 140:248221
TITLE: Myrothecium sp. transformation and expression system
INVENTOR(S): Jonniaux, Jean-Luc; Valepyn, Emmanuel; Corbisier,

Anne-Marie; Dauvrin, Thierry
PATENT ASSIGNEE(S): Puratos Naamloze Vennootschap, Belg.
SOURCE: PCT Int. Appl., 83 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004020611	A1	20040311	WO 2003-BE143	20030829
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003258395	A1	20040319	AU 2003-258395	20030829
EP 1539926	A1	20050615	EP 2003-790573	20030829
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				

PRIORITY APPLN. INFO.:
US 2002-407843P P 20020830
WO 2003-BE143 W 20030829

AB The present invention is related to a transformation and an expression system in which Myrothecium sp. host cells are used to express homologous or heterologous proteins or are used to genetically engineer metabolic pathways.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2003:678997 CAPLUS
DOCUMENT NUMBER: 139:192491
TITLE: Methods for optimized codon usage for plant polypeptide synthesis in filamentous fungi
INVENTOR(S): Taira, Rikako; Tsutsumi, Noriko; Terui, Yuri; Takagi, Shinobu
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003070957	A2	20030828	WO 2003-DK108	20030219
WO 2003070957	A3	20031224		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003206684	A1	20030909	AU 2003-206684	20030219

PRIORITY APPLN. INFO.:
DK 2002-263 A 20020220
DK 2002-871 A 20020607
WO 2003-DK108 W 20030219

AB The present invention provides altered codon usage in genes encoding plant polypeptides for increased heterologous expression and production of plant

polypeptides of interest in filamentous fungi host cells. This invention evaluates the frequency of and impact of codon mutations upon heterologous expression of plant polypeptide genes. Mutagenesis of plant DNA sequences, creation of vector constructs, and genetic transfer of these mutant constructs to fungal hosts are provided.

L3 ANSWER 8 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2002:736355 CAPLUS
DOCUMENT NUMBER: 137:246620
TITLE: Improved fermentation process
INVENTOR(S): Olsen, Hans Sejr; Pedersen, Sven; Beckerich, Robert; Veit, Christopher; Felby, Claus
PATENT ASSIGNEE(S): Novozymes A/S, Den.; Novozymes North America, Inc.
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2002074895	A2	20020926	WO 2002-DK179		20020319
WO 2002074895	A3	20030612			
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW					
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG					
EP 1373539	A2	20040102	EP 2002-708252		20020319
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR					
US 2004115779	A1	20040617	US 2003-472256		20030919
PRIORITY APPLN. INFO.:			US 2001-277383P	P	20010319
			US 2001-277384P	P	20010319
			US 2001-304380P	P	20010710
			WO 2002-DK179	W	20020319

AB The present invention relates to an improved process for producing a fermentation product. Thus, ethanol fermentation of whole corn mash by Saccharomyces cerevisiae was enhanced by the addition of glucoamylase and β-glucanase.

L3 ANSWER 9 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 1998:197593 CAPLUS
DOCUMENT NUMBER: 128:279553
TITLE: Cloning and expression of metalloproteinase and other heterologous protein genes from fungi
INVENTOR(S): Lehmbeck, Jan
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.; Lehmbeck, Jan
SOURCE: PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9812300	A1	19980326	WO 1997-DK397	19970919
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,				

GN, ML, MR, NE, SN, TD, TG

CN 1179178	A	19980415	CN 1996-192700	19960320
AU 9742008	A1	19980414	AU 1997-42008	19970919
CN 1230986	A	19991006	CN 1997-198045	19970919
EP 956338	A1	19991117	EP 1997-939990	19970919
EP 956338	B1	20051221		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2001500381	T2	20010116	JP 1998-514203	19970919
AT 313620	E	20060115	AT 1997-939990	19970919
US 6352841	B1	20020305	US 1999-252509	19990218
PRIORITY APPLN. INFO.:			DK 1996-1024 A	19960919
			WO 1997-DK397 W	19970919

AB The present invention relates to novel host cells and to methods of producing proteins. More specifically the invention relates to a host cell useful for the expression of heterologous proteins, in which the host cell has been genetically modified in order to express significantly reduced levels of a metalloprotease and an alkaline protease. Moreover the invention relates to a method of producing a heterologous protein, which method comprises cultivating the host cell in a suitable growth medium, followed by recovery of the desired protein. The invention was used to producing neutral metalloprotease I and alkaline protease proteins, either individually or in combination. Thus plasmid vectors such as pPSO5 were constructed which contains the Fusarium oxysporum metalloprotease gene p45 or the Aspergillus oryzae neutral metalloprotease I gene. The genes are cloned in Saccharomyces cerevisiae, preferably, but can also be cloned in Acremonium, Aspergillus, Candida, Cochliobolus, Endothia, Fusarium, Humicola, Neurospora, **Rhizomucor**, Rhizopus, Thermomyces, Trichoderma, Podospora, Pyricularia, and Penicillium. In addition to the proteinases, other therapeutic proteins can be prepared such as insulin, somatotropin, glucagon, somatostatin, interferon, erythropoietin, TPO, PDGF, factor VII, factor VIII, urokinase, chymosin, tissue plasminogen activator, or serum albumin. Fungal enzymes also may produced. These include α -**amylase**, β-amylase, glucoamylase, β-galactosidase, cellulolytic enzymes, lipolytic enzymes, xylanolytic enzymes, proteolytic enzymes, oxidoreductase (.e.g. peroxidase or laccase), pectinase, or a cutinase.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 10 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 5

ACCESSION NUMBER: 1994:536684 CAPLUS
DOCUMENT NUMBER: 121:136684
TITLE: Recombinant lipase and **alpha-amylase** variants resistant to inactivation by peroxidase systems and use of the enzymes in detergents compositions
INVENTOR(S): Nielsen, Egon; Rasmussen, Grethe; Halkier, Torben
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9414951	A1	19940707	WO 1993-DK441	19931222
W: BR, FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 675949	A1	19951011	EP 1994-904145	19931222
EP 675949	B1	20041020		
R: AT, BE, DE, DK, ES, FR, GB, IT, NL				
JP 08504586	T2	19960521	JP 1993-514708	19931222
BR 9307718	A	19990908	BR 1993-7718	19931222
AT 280220	E	20041115	AT 1994-904145	19931222
US 5731280	A	19980324	US 1995-448540	19950615
FI 9503128	A	19950622	FI 1995-3128	19950622
PRIORITY APPLN. INFO.:			DK 1992-1542 A	19921223
			WO 1993-DK441 W	19931222

AB The present invention relates to lipase and α -**amylase** variants, stabilized towards the inactivation caused by peroxidase systems, in which variants a naturally occurring tyrosine residue has been deleted or substituted with a different amino acid residue at one or more positions. The invention also relates to a method of stabilizing a lipase or an α -**amylase** towards the inactivation caused by peroxidase systems, and detergent compns. comprising a lipase and/or an α -**amylase** variant of the invention. Numerous detergent compns. were given.

L3 ANSWER 11 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 1993:74769 CAPLUS
DOCUMENT NUMBER: 118:74769
TITLE: Manufacture of heterologous heme proteins with filamentous fungi
INVENTOR(S): Andersen, Henrik Dalboge; Jensen, Ejner Bech; Welinder, Karen Gjesing
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: Eur. Pat. Appl., 17 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 505311	A2	19920923	EP 1992-610017	19920320
EP 505311	A3	19930728		
EP 505311	B1	20000607		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
CA 2106485	AA	19920923	CA 1992-2106485	19920320
WO 9216634	A1	19921001	WO 1992-DK88	19920320
W: BR, CA, FI, JP, US				
BR 9205802	A	19940628	BR 1992-5802	19920320
JP 06506108	T2	19940714	JP 1992-506829	19920320
JP 3343117	B2	20021111		
AT 193727	E	20000615	AT 1992-610017	19920320
ES 2148168	T3	20001016	ES 1992-610017	19920320
FI 111957	B1	20031015	FI 1993-4135	19930921
US 5744323	A	19980428	US 1994-315671	19940930
US 5958724	A	19990928	US 1997-858933	19970520
GR 3034287	T3	20001229	GR 2000-401974	20000830

PRIORITY APPLN. INFO.:

EP 1991-610022	A	19910322
EP 1992-610017	A	19920320
WO 1992-DK88	W	19920320
US 1993-119077	B1	19930915
US 1994-315671	A3	19940930

AB Heterologous heme proteins are manufactured with filamentous fungi containing a vector comprising a gene for the heme protein fused to a preregion facilitating secretion of the protein. The cDNA for Coprinus cinereus peroxidase was cloned and inserted into a plasmid downstream of the TAKA amylase promoter and signal sequence of Aspergillus oryzae. A. oryzae transformed with this vector and cultured in medium containing hemin and Glanapon DG160 surfactant produced 1480 U peroxidase/mL after 72 h.

L3 ANSWER 12 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 1990:173629 CAPLUS
DOCUMENT NUMBER: 112:173629
TITLE: Molecular cloning in Aspergillus
INVENTOR(S): Woldike, Helle Fabricius
PATENT ASSIGNEE(S): Novo Industri A/S, Den.
SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 8901969	A1	19890309	WO 1988-DK145	19880902
W: DK, JP, US				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
EP 383779	A1	19900829	EP 1988-908169	19880902
EP 383779	B1	19931208		
EP 383779	B2	20000531		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
JP 04503150	T2	19920611	JP 1988-507560	19880902
JP 2703598	B2	19980126		
AT 98299	E	19931215	AT 1988-908169	19880902
DK 9000531	A	19900301	DK 1990-531	19900301
US 5252726	A	19931012	US 1992-859596	19920323
PRIORITY APPLN. INFO.:			DK 1987-4609 A	19870904
			DK 1987-5126 A	19870929
			EP 1988-908169 A	19880902
			WO 1988-DK145 W	19880902
			US 1990-469509 B1	19900313

AB A procedure is disclosed for mol. cloning in Aspergillus, especially A. niger. The fungus is transformed with a plasmid that is capable of integrating into the host genome in 1 or more copies and which contains the following: (1) promoter and upstream activation sequence of an A. niger amylase gene; (2) a suitable marker for selection of transformants; and (3) a gene coding for a desired protein product. The gene for the desired product may be provided with a preregion to allow secretion of the protein product into the culture medium. The cloning procedure can be used for the industrial production of many different products by the recombinant Aspergillus. Examples of such products include chymosin or prochymosin and other rennets, proteases, lipases, and amylases. In 1 embodiment of the invention, the gene for aspartic protease of **Rhizomucor** miehei was cloned and expressed in both A. niger and A. oryzae.

L3 ANSWER 13 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 1989:167424 CAPLUS
DOCUMENT NUMBER: 110:167424
TITLE: High level expression of recombinant genes in Aspergillus oryzae
AUTHOR(S): Christensen, Tove; Woeldike, Helle; Boel, Esper; Mortensen, Steen B.; Hjortshoej, Kirsten; Thim, Lars; Hansen, Mogens T.
CORPORATE SOURCE: Novo Res. Inst., Bagsvaerd, DK-2880, Den.
SOURCE: Bio/Technology (1988), 6(12), 1419-22
CODEN: BTCHDA; ISSN: 0733-222X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A method was developed for transformation of A. oryzae by modification of a transformation system for Aspergillus nidulans. The argB and amdS genes of A. nidulans were used as selectable markers. The amdS gene codes for an acetamidase and enables A. oryzae to grow on acetamide as its sole nitrogen source. Prototrophic transformants can be selected in argB mutant strains by transformation with a functional argB gene. An . **alpha.-amylase** gene was cloned from a high yielding strain of A. oryzae and its promoter was used to direct the expression of recombinant genes in A. oryzae. The aspartic proteinase gene of **Rhizomucor** miehi was cloned and expressed in A. oryzae and was secreted with yields in excess of 3 g/L. The proteinase was slightly overglycosylated, but this did not alter the specific activity of the enzyme. The amts. of heterologous protein obtained make this system attractive for even moderately priced industrial enzymes.

L3 ANSWER 14 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:534312 CAPLUS
DOCUMENT NUMBER: 141:67294
TITLE: Cloning, purification and characterization of thermostable α -**amylase** from **Rhizomucor** pusillus, and use in liquefying starch, production of alcohol, brewing and baking
INVENTOR(S): Tang, Lan; Wu, Wenping; Duan, Junxin; Johannesen, Pia Francke
PATENT ASSIGNEE(S): Novozymes A/S, Den.

SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004055178	A1	20040701	WO 2003-DK882	20031216
WO 2004055178	C2	20041007		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003287900	A1	20040709	AU 2003-287900	20031216
EP 1576152	A1	20050921	EP 2003-779740	20031216
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
US 2006051849	A1	20060309	US 2005-539396	20050616

PRIORITY APPLN. INFO.: DK 2002-1928 A 20021217
WO 2003-DK882 W 20031216

AB The present inventors have successfully isolated a gene from **Rhizomucor** pusillus encoding an **alpha-amylase** which they have denoted AM782, they have successfully introduced the encoding gene into a recombinant industrial filamentous fungal expression system, and produced the **alpha-amylase.** Characterization of the amylase has shown it to be a highly thermoacidophilic **alpha-amylase** which has a highly interesting activity as demonstrated by the sugar profile from maltodextrin hydrolysis by amylase AM782. The amylase AM782 can work at a very high temperature, at least up to 70°. The amylase AM782 has a very fast reaction speed; when compared at the same dosage with Fungamyl 800 L, the amylase AM782 can achieve in about 3 h, what takes Fungamyl 24 to 48 h. Purification and characterization of the **alpha-amylase** from **Rhizomucor** pusillus NN046782 is described. Cloning of the gene encoding the AM782 **alpha-amylase** of **Rhizomucor** pusillus NN046782 and subcloning and heterologous expression of AM782 amylase is also described. The thermoacidophilic **alpha-amylase** of the invention can be used in starch conversion for liquefaction and saccharification, for liquefying starch in a high maltose syrup, for producing alc., for textile desizing, and for brewing and baking.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 15 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:402732 CAPLUS
DOCUMENT NUMBER: 140:374008
TITLE: Aspartic proteinase-deficient filamentous fungi for improved production of heterologous proteins
INVENTOR(S): Berka, Randy M.; Hayenga, Kirk J.; Lawlis, Virgil B.; Ward, Michael
PATENT ASSIGNEE(S): Genencor International, Inc., USA
SOURCE: U.S., 28 pp., Cont. of U.S. 5,840,570.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6509171	B1	20030121	US 1998-48473	19980326

CA 1333777	A1	19950103	CA 1989-604556	19890630
WO 9000192	A1	19900111	WO 1989-US2891	19890701
W: FI, JP				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
EP 429490	A1	19910605	EP 1989-908939	19890701
EP 429490	B1	19950125		
EP 429490	B2	20040922		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
AT 117720	E	19950215	AT 1989-908939	19890701
US 5840570	A	19981124	US 1994-345018	19941123
PRIORITY APPLN. INFO.:			US 1988-214237	B1 19880701
			US 1992-931123	B1 19920817
			US 1994-345018	A1 19941123
			WO 1989-US2891	W 19890701

AB This invention relates to novel mutant filamentous fungi which are deficient in the gene for the corresponding aspartic proteinase. Thus, genomic DNA encoding aspergillopepsin A from Aspergillus awamori was cloned and characterized; a gene replacement strategy similar to that described in Mol. Cell. Biol. (volume 5, pp. 1714-1721, 1985) was used to generate strains of A. awamori that were specifically deficient in the production of aspergillopepsin. Greater production of recombinant chymosin is observed in deficient mutants of A. awamori in comparison to wild-type, probably as a result of decreased degradation Thus, aspartic proteinase-deficient organisms are useful production hosts in the production of heterologous polypeptides such as chymosin.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 16 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1996:483910 CAPLUS

DOCUMENT NUMBER: 125:160364

TITLE: Vector constructs for Aspergillus recombinant protein production, Humicola lanuginosa lipase cDNA sequence, and industrial applications

INVENTOR(S): Boel, Esper; Christensen, Tove; Woldike, Helle

PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.

SOURCE: U.S., 51 pp., Cont. of U.S. Ser. No. 236,605, abandoned.
CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5536661	A	19960716	US 1994-208092	19940307
US 5766912	A	19980616	US 1994-230170	19940420
US 5863759	A	19990126	US 1995-463957	19950605
US 5965384	A	19991012	US 1995-463172	19950605
US 5874558	A	19990223	US 1996-650086	19960517
PRIORITY APPLN. INFO.:			US 1987-24342	B2 19870310
			US 1988-236605	B1 19880825
			DK 1986-1226	A 19860317
			DK 1987-4500	A 19870828
			DK 1987-6560	A 19871215
			DK 1988-2054	A 19880415
			US 1992-954371	B3 19920930
			US 1994-208092	A1 19940307
			US 1994-230170	A3 19940420
			US 1995-435557	B3 19950505

AB A process for expression of a protein product in Aspergillus oryzae is disclosed. The process comprises transforming Aspergillus oryzae with a vector system comprising DNA-sequences encoding functions facilitating gene expression, a suitable marker for selection of transformants, and a DNA-sequence encoding the desired protein product. The process enables industrial production of many different polypeptides and proteins in A. oryzae. Examples of such products are chymosin or prochymosin and other rennets, proteases, lipases and amylases. Also disclosed is an effective promoter for expression of a protein in Aspergillus. A preferred promoter

is the TAKA-amylase promoter or functional parts thereof. There is also provided a process for the production of a recombinant Humicola lipase. The recombinant Humicola lipase from A. oryzae differs from the native lipase in having a greater glycosylation and in exhibiting an improved thermostability.

L3 ANSWER 17 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1995:785012 CAPLUS
DOCUMENT NUMBER: 123:167718
TITLE: Retransformation of filamentous fungi and use for protein production with improved yields
INVENTOR(S): Reeh, Solvejg
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9517513	A1	19950629	WO 1994-DK488	19941222
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN				
RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9512726	A1	19950710	AU 1995-12726	19941222
PRIORITY APPLN. INFO.:			DK 1993-1737 A	19931223
			WO 1994-DK488 W	19941222

AB The invention relates to a process for producing a protein in a filamentous fungus in improved yields, the process comprising (a) transforming a suitable filamentous fungus with a first recombinant DNA construct comprising a DNA sequence encoding said protein as well as with a first DNA sequence coding for a suitable marker for the selection of transformants, (b) retransforming said transformant with a second recombinant DNA construct which comprises a DNA sequence encoding said protein or another protein as well as with a second DNA sequence coding for a suitable marker for the selection of transformants, and (c) culturing the retransformed filamentous fungus in a suitable culture medium under conditions permitting the production of the protein. The first or second DNA construct, and/or the first or second DNA sequence may comprise a stretch of DNA homologous to the host. The invention is exemplified in the production of different proteins using various filamentous fungal hosts.

L3 ANSWER 18 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1995:804430 CAPLUS
DOCUMENT NUMBER: 123:220284
TITLE: Promoters for expressing protein products in Aspergillus
INVENTOR(S): Boel, Esper; Christensen, Tove; Woeldike, Helle Fabricius
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: Dan., 59 pp.
CODEN: DAXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Danish
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DK 170118	B1	19950529	DK 1991-912	19910515
DK 9100912	A	19910515		
PRIORITY APPLN. INFO.:			DK 1991-912	19910515

AB The promoters are the Aspergillus oryzae TAKA-amylase promoter or a functional part thereof, optionally preceded by the naturally associated

upstream activation sequences. The A. oryzae TAKA-amylase gene was cloned. An Aspergillus expression vector designed to obtain secretion of **Rhizomucor** miehei lipase under control of the A. oryzae TAKA-amylase promotor was constructed. A. oryzae was transformed with this vector and cultured to obtain the enzyme.

L3 ANSWER 19 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:6094 CAPLUS
DOCUMENT NUMBER: 112:6094
TITLE: Manufacture of Humicola lanuginosa lipase by recombinant Aspergillus
INVENTOR(S): Boel, Esper; Huge-Jensen, Ida Birgitte
PATENT ASSIGNEE(S): Novo Industri A/S, Den.
SOURCE: Eur. Pat. Appl., 28 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 305216	A1	19890301	EP 1988-307980	19880826
EP 305216	B1	19950802		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
DK 8804760	A	19890419	DK 1988-4760	19880826
DK 165640	B	19921228		
DK 165640	C	19930601		
ES 2076939	T3	19951116	ES 1988-307980	19880826
JP 01157383	A2	19890620	JP 1988-212641	19880829
JP 04038394	B4	19920624		
DK 9101775	A	19911025	DK 1991-1775	19911025
US 5766912	A	19980616	US 1994-230170	19940420
US 5965384	A	19991012	US 1995-463172	19950605
US 5874558	A	19990223	US 1996-650086	19960517

PRIORITY APPLN. INFO.:

DK 1987-4500	A	19870828
DK 1987-6560	A	19871215
DK 1988-2054	A	19880415
DK 1986-1226	A	19860317
US 1987-24342	B2	19870310
US 1988-236605	B1	19880825
US 1992-954371	B3	19920930
US 1994-230170	A3	19940420
US 1995-435557	B3	19950505

AB Humicola lanuginosa lipase (I) is manufactured by culturing a transformed Aspergillus host, e.g. A. niger, A. oryzae, and recovering I from the culture medium. Recombinant I has better thermostability than comparable native lipase (II), is more resistant to proteolytic degradation than II, and has a different pattern of glycosylation from II. Plasmid p960 containing the TAKA-amylase promoter from A. oryzae, I gene from Humicola lanuginosa, and AMG terminator from A. niger was constructed and transformed into A. oryzae IFO 4177 by cotransformation with P3SR2 containing the amdS gene from A. nidulans. The A. oryzae transformants were cultured in 40% soybean meal plus glucose. I was recovered from the culture medium by ultrafiltration and freeze drying. I contained N-acetylglucosamine 1.2, mannose 8.6, and galactose 3.3 mol/mol I compared to 1.2, 5.7, and 0 mol/mol II, resp. I had better thermostability than II at pH 5-10 at 55° and 60°, resp. and I was less susceptible to Bacillus protease than II.

L3 ANSWER 20 OF 26 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1988:418172 CAPLUS
DOCUMENT NUMBER: 109:18172
TITLE: Process for the production of protein products in Aspergillus oryzae and promoters for use in Aspergillus
INVENTOR(S): Boel, Esper; Christensen, Tove; Woldike, Helle Fabricius
PATENT ASSIGNEE(S): Novo Industri A/S, Den.
SOURCE: Eur. Pat. Appl., 40 pp.

CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 238023	A2	19870923	EP 1987-103806	19870316
EP 238023	A3	19890222		
EP 238023	B1	19931222		
EP 238023	B2	20021002		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
FI 8701144	A	19870918	FI 1987-1144	19870316
FI 108147	B1	20011130		
EP 489718	A1	19920610	EP 1992-104421	19870316
EP 489718	B1	20041110		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 98993	E	19940115	AT 1987-103806	19870316
ES 2061446	T3	19941216	ES 1987-103806	19870316
AT 282093	E	20041115	AT 1992-104421	19870316
EP 1502952	A2	20050202	EP 2004-26492	19870316
EP 1502952	A3	20050511		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
DK 8701353	A	19871027	DK 1987-1353	19870317
DK 169134	B1	19940822		
JP 62272988	A2	19871127	JP 1987-60276	19870317
JP 06065316	B4	19940824		
JP 10276787	A2	19981020	JP 1997-356759	19870317
JP 2003153696	A2	20030527	JP 2002-265161	19870317
JP 07051067	A2	19950228	JP 1994-7137	19940126
JP 3005618	B2	20000131		
US 5766912	A	19980616	US 1994-230170	19940420
US 5965384	A	19991012	US 1995-463172	19950605
US 5874558	A	19990223	US 1996-650086	19960517
FI 2001001797	A	20010912	FI 2001-1797	20010912
FI 112376	B1	20031128		

PRIORITY APPLN. INFO.:		
DK 1986-1226	A	19860317
US 1987-24342	B2	19870310
EP 1987-103806	A	19870316
JP 1987-60276	A3	19870317
JP 1997-356759	A3	19870317
DK 1987-4500	A	19870828
DK 1987-6560	A	19871215
DK 1988-2054	A	19880415
US 1988-236605	B1	19880825
EP 1992-104421	A3	19920313
US 1992-954371	B3	19920930
US 1994-230170	A3	19940420
US 1995-435557	B3	19950505

AB Plasmids are constructed for cloning foreign genes in protoplasts of A. oryzae. In plasmid p686, the acid proteinase gene of **Rhizomucor** miehei was placed under the control of the glucoamylase promoter of A. niger; in p777, the acid proteinase gene was regulated by the A. oryzae TAKA-amylase promoter. In plasmid p787, the lipase gene of R. miehei was under the control of the TAKA-amylase promoter, and in plasmid pToC56, the calf prochymosin gene was controlled by this promoter.

L3 ANSWER 21 OF 26 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-06714 BIOTECHDS
TITLE: Expressing a protein product in Aspergillus oryzae by providing a recombinant DNA cloning vector system capable of integration into the genome of an A. oryzae host and culturing the transformed A. oryzae host in a culture medium; technique for production of a recombinant protein in Aspergillus oryzae
AUTHOR: BOEL E; CHRISTENSEN T; WOLDIKE H F
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: EP 1502952 2 Feb 2005
APPLICATION INFO: EP 1987-26492 16 Mar 1987

PRIORITY INFO: DK 1986-1226 17 Mar 1986; DK 1986-1226 17 Mar 1986
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-114422 [13]
AN 2005-06714 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Expressing a protein product in Aspergillus oryzae comprises: (1) providing a recombinant DNA cloning vector system capable of integration into the genome of an Aspergillus oryzae host; (2) transforming the Aspergillus oryzae host which does not harbor a functional gene for the chosen selection marker with the recombinant DNA cloning vector system from step (1); and (3) culturing the transformed Aspergillus oryzae host in a suitable culture medium.

DETAILED DESCRIPTION - Expressing a protein product in Aspergillus oryzae comprises: (1) providing a recombinant DNA cloning vector system capable of integration into the genome of an Aspergillus oryzae host in one or more copies and comprising DNA-sequences encoding functions facilitating gene expression, a suitable marker for selection of transformants, and a DNA-sequence encoding the desired protein product; (2) transforming the Aspergillus oryzae host which does not harbor a functional gene for the chosen selection marker with the recombinant DNA cloning vector system from step (1); and (3) culturing the transformed Aspergillus oryzae host in a suitable culture medium. INDEPENDENT CLAIMS are also included for the following: (1) a process for producing a protein product in Aspergillus oryzae, where an Aspergillus oryzae strain being transformed with a recombinant DNA cloning vector system is cultured in a suitable culture medium and the product is recovered from the culture medium; and (2) a promoter suitable for expression of a protein product in Aspergillus, which is the TAKA-amylase promoter or its functional parts optionally preceded by upstream activating sequences.

BIOTECHNOLOGY - Preferred Method: Expressing a protein product in Aspergillus oryzae comprises: (1) providing a recombinant DNA cloning vector system capable of integration into the genome of an Aspergillus oryzae host in one or more copies and comprising DNA-sequences encoding functions facilitating gene expression, a suitable marker for selection of transformants, and a DNA-sequence encoding the desired protein product; (2) transforming the Aspergillus oryzae host which does not harbor a functional gene for the chosen selection marker with the recombinant DNA cloning vector system from step (1); and (3) culturing the transformed Aspergillus oryzae host in a suitable culture medium. The DNA-sequences encoding functions facilitating gene expression comprises a promoter, transcription initiation sites, and transcription terminator and polyadenylation functions. The promoter is preceded by upstream activating sequences. The selection marker is derived from the gene for Aspergillus nidulans or Aspergillus niger argB, Aspergillus nidulans trpC, Aspergillus nidulans amdS, Neurospora crassae Pyr4 or DHFR. The selection marker is the ArgB gene derived from Aspergillus nidulans or Aspergillus niger or the amdS gene derived from Aspergillus nidulans. The promoter and upstream activating sequences are derived from a gene encoding an extracellular or intracellular protein, such as an amylase, a glucoamylase, a protease, a lipase, a cellulase or a glycolytic enzyme. The promoter and upstream activating sequences are derived from the gene of Aspergillus oryzae TAKA amylase, **Rhizomucor** miehei aspartic proteinase, Aspergillus niger neutral **alpha-amylase**, Aspergillus niger acid stable **alpha-amylase**, Aspergillus niger glucoamylase or **Rhizomucor** miehei lipase. The promoter is the Aspergillus oryzae TAKA amylase promoter or its functional parts. The promoter and upstream activating sequences have the sequence GTCGACGCATTCCGAATACGAGGCCTGATTAATGATTACATACGCCTCCGGGTAGTAGACCGAG CAGCCGAGCCAGTTCAGCGCCTAAAACGCCTTATACAATTAAGCAGTTAAAGAAGTTAGAATCTACGCTTAAA AAGCTACTTAAAAATCGATCTCGCAGTCCCGATTCGCCTATCAAAACCAGTTTAAATCAACTGATTAAAGGTG CCGAACGAGCTATAAATGATATAACAATATTAAAGCATTAATTAGAGCAATATCAGGCCGCGCACGAAAGGCA ACTTAAAAAGCGAAAGCGCTCTACTAAACAGATTACTTTTGAAAAAGGCACATCAGTATTTAAAGCCCGAATC CTTATTAAGCGCCGAAATCAGGCAGATAAAGCCATACAGGCAGATAGACCTCTACCTATTAAATCGGCTTCTA GGCGCGCTCCATCTAAATGTTCTGGCTGTGGTGTACAGGGGCATAAAATTACGCACTACCCGAATCGATAGAA CTACTCATTTTTATATAGAAGTCAGAATTCATAGTGTTTTGATCATTTTAAATTTTTATATGGCGGGTGGTGG GCAACTCGCTTGCGCGGGCAACTCGCTTACCGATTACGTTAGGGCTGATATTTACGTGAAAATCGTCAAGGGA TGCAAGACCAAAGTAGTAAAACCCCGGAAGTCAACAGCATCCAAGCCCAAGTCCTTCACGGAGAAACCCCAGC GTCCACATCACGAGCGAAGGACCACCTCTAGGCATCGGACGCACCATCCAATTAGAACAGCAAAGCGAAACAG CCCAAGAAAAAGGTCGGCCCGTCGGCCTTTTCTGCAACGCTGATCACGGGCAGCGATCCAACCAACACCCTCC

AGAGTGACTAGGGGCGGAAATTTAAAGGGATTAATTTCCACTCAACCACAAATCACAGTCGTCCCCGGTATTG
TCCTGCAGAATGCAATTTAAACTCTTCTGCGAATCGCTTGGATTCCCCGCCCCTAGTCGTAGAGCTTAAAGTA
TGTCCCTTGTCGATGCGATGTATCACAACATATAAATACTAGCAAGGGATGCCATGCTTGGAGGATAGCAACC
GACAACATCACATCAAGCTCT CCCTTCTCTGAACAATAAAC CCCACAG or its functionally
equivalent nucleotide sequence. The promoter and upstream activating
sequences may also have the sequence AGATCTGCCCTTATAAATCTCCTAGTCTGATCGTCG
ACGCATTCCGAATACGAGGCCTGATTAATGATTACATACGCCTCCGGGTAGTAGACCGAGCAGCCGAGCCAGT
TCAGCGCCTAAAACGCCTTATACAATTAAGCAGTTAAAGAAGTTAGAATCTACGCTTAAAAAGCTACTTAAAA
ATCGATCTCGCAGTCCCGATTCGCCTATCAAAACCAGTTTAAATCAACTGATTAAAGGTGCCGAACGAGCTAT
AAATGATATAACAATATTAAAGCATTAATTAGAGCAATATCAGGCCGCGCACGAAAGGCAACTTAAAAAGCGA
AAGCGCTCTACTAAACAGATTACTTTTGAAAAAGGCACATCAGTATTTAAAGCCCGAATCCTTATTAAGCGCC
GAAATCAGGCAGATAAAGCCATACAGGCAGATAGACCTCTACCTATTAAATCGGCTTCTAGGCGCGCTCCATC
TAAATGTTCTGGCTGTGGTGTACAGGGGCATAAAATTACGCACTACCCGAATCGATAGAACTACTCATTTTTA
TATAGAAGTCAGAATTCATAGTGTTTTGATCATTTTAAATTTTTATATGGCGGGTGGTGGGCAACTCGCTTGC
GCGGGCAACTCGCTTACCGATTACGTTAGGGCTGATATTTACGTGAAAATCGTCAAGGGATGCAAGACCAAAG
TAGTAAAACCCCGGAAGTCAACAGCATCCAAGCCCAAGTCCTTCACGGAGAAACCCCAGCGTCCACATCACGA
GCGAAGGACCACCTCTAGGCATCGGACGCACCATCCAATTAGAAGCAGCAAAGCGAAACAGCCCAAGAAAAAG
GTCGGCCCGTCGGCCTTTTCTGCAACGCTGATCACGGGCAGCGATCCAACCAACACCCTCCAGAGTGACTAGG
GGCGGAAATTTAAAGGGATTAATTTCCACTCAACCACAAATCACAGTCGTCCCCGGTATTGTCCTGCAGAATG
CAATTTAAACTCTTCTGCGAATCGCTTGGATTCCCCGCCCCTAGTCGTAGAGCTTAAAGTATGTCCCTTGTCG
ATGCGATGTATCACAACATATAAATACTAGCAAGGGATGCCATGCTTGGAGGATAGCAACCGACAACATCACA
TCAAGCTCTCCCTTCTCTGAACAATAAAC CCCACAGAAGGCATTT or its functionally
equivalent nucleotide sequence. The sequence is preceded by the 1.05 kb
unsequenced upstream region from position 0-1.05 in plasmid pTAKA 17. The
vector system further comprises a pre-region providing for secretion of
the expressed product into the culture medium. The pre-region is derived
from a glucoamylase or an amylase gene from an Aspergillus species, an
amylase gene from a Bacillus species, a lipase or proteinase gene from
Rhizomucor miehei, the gene for the a-factor from E. cerevisiae
or the calf prochymosin gene. The pre-region is derived from the gene for
A. oryzae TAKA amylase, A. niger neutral **alpha-amylase**
, A. niger acid-stable **alpha-amylase**, Bacillus
licheniformis **alpha-amylase**, the maltogenic amylase
from Bacillus NCIB 11837, B. stearothermophilus a-amylase or B.
licheniformis subtilisin. The pre-region is the TAKA-amylase pre-region
with the sequence ATGATGGTCGCGTGGTGGTCTCTATTTCTGTACGGCCTTCAGGTCGCGGCACCTG
CTTTGGCT with corresponding sequence Met-Met-Val-Ala-Trp-Trp-Ser-Leu-Phe-
Leu-Tyr-Gly-Leu-Gln-Val-Ala-Ala-Pro-Ala-Leu-Ala. The vector system
comprises two vectors, where one contains the selection marker and the
other contains DNA-sequences encoding functions facilitating gene
expression and a DNA sequence encoding the desired protein product.

USE - The method is useful in expressing a protein product in
Aspergillus oryzae (claimed). (42 pages)

L3 ANSWER 22 OF 26 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-12650 BIOTECHDS
TITLE: Producing a mammalian trypsin, for use in the detergent, leather, pharmaceutical, food and dairy industries, comprises cultivating a Fusarium venenatum host strain in a culture medium for expression and secretion of the mammalian trypsin; recombinant enzyme production via plasmid expression in host cell for use in food and pharmaceutical industry
AUTHOR: BERKA R; BROWN K
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2004043455 4 Mar 2004
APPLICATION INFO: US 2003-651790 29 Aug 2003
PRIORITY INFO: US 2003-651790 29 Aug 2003; US 2002-407170 30 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-225702 [21]
AN 2004-12650 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Producing a mammalian trypsin comprises cultivating a Fusarium venenatum host strain comprising a nucleic acid construct in a culture medium for expression and secretion of the mammalian trypsin into the medium, and recovering the mammalian trypsin from the medium, is new

DETAILED DESCRIPTION - Producing a mammalian trypsin comprises cultivating a Fusarium venenatum host strain comprising a nucleic acid construct in a culture medium for expression and secretion of the mammalian trypsin into the medium, and recovering the mammalian trypsin

from the medium. The F. venenatum host strain comprises a nucleic acid construct comprising a nucleic acid sequence encoding the mature coding sequence of a mammalian trypsin operably linked to nucleotides 58-129 of a sequence of 998 bp (I), fully defined in the specification, encoding the signal peptide and propeptide of Fusarium oxysporum trypsinogen. INDEPENDENT CLAIMS are also included for: (1) a nucleic acid construct comprising a nucleic acid sequence encoding the mature coding sequence of a mammalian trypsin operably linked to nucleotides 58-129 of the sequence of (I), encoding the signal peptide and propeptide of F. oxysporum trypsinogen; (2) a recombinant expression vector comprising the nucleic acid construct; and (3) a recombinant F. venenatum host strain comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Host Strain: The F. venenatum host strain is F. venenatum ATCC 20334, a morphological mutant of F. venenatum ATCC 20334, or a trichothecene-deficient and/or a cyclohexadepsipeptide-deficient F. venenatum strain. Preferred Nucleic Acid Construct: The nucleic acid sequence encoding the mature coding sequence of the mammalian trypsin is nucleotides 75-744 of a sequence of 897 bp, fully defined in the specification. The mature coding sequence of the mammalian trypsin encodes amino acids 25-247 of a sequence of 247 amino acids, fully defined in the specification. Preferred Method: In producing a mammalian trypsin, the nucleic acid construct further comprises a promoter obtained from a gene consisting of an Aspergillus oryzae TAKA amylase, **Rhizomucor** miehei aspartic proteinase, Aspergillus niger neutral **alpha-amylase**, A. niger acid stable **alpha-amylase**, A. niger or Aspergillus awamori glucoamylase (glaA), R. miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase, Aspergillus nidulans acetamidase, A. oryzae acetamidase, F. oxysporum trypsin-like enzyme, F. venenatum AMG, F. venenatum Daria, or F. venenatum Quinn gene. The nucleic acid construct further comprises a terminator obtained from a gene selected consisting of an A. oryzae TAKA amylase, A. niger glucoamylase, A. nidulans anthranilate synthase, A. niger alpha-glucosidase, or F. oxysporum trypsin-like protease. Preferably, the nucleic acid construct further comprises a promoter and a terminator obtained from a F. oxysporum trypsin-like gene. The mammalian trypsin is a bovine, cow, dog, human, mouse, pig, or rat trypsin.

USE - The nucleic acid construct, vector and F. venenatum host strain are useful in producing mammalian trypsins for detergent, leather, chemical, agricultural, pharmaceutical, food and dairy industries.

EXAMPLE - Protoplasts were prepared by inoculating 100 ml of YEPG medium with 4 x 107 spores of Fusarium venenatum MLY-3 and incubating for 16 hours at 24 degreesC and 150 rpm. A 100 micrograms quantity of vector pRaMB58 containing a hybrid coding region comprising the Fusarium oxysporum trypsin signal peptide and propeptide region and the mature porcine trypsin sequence was added to a 50 ml sterile polypropylene tube and 2 ml of protoplasts were added to the tube, mixed gently and incubated. Four F. venenatum transformants were obtained with pRaMB58. The transformants were picked directly from the selection plates into 125 ml shake flasks containing 25 ml of M400 medium and incubated at 28 degreesC for 6 days. Broth samples from transformants were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Results showed that the transformants secrete a prominent polypeptide with an apparent molecular weight of approximately 23 kDa, which is the expected size of porcine trypsin.(21 pages)

L3 ANSWER 23 OF 26 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1995-10491 BIOTECHDS
TITLE: Aspergillus japonicus-type cells expressing heterologous enzyme;
fungus recombinant lipase, endo-1,4-beta-D-xylanase and cellulase over-production by expression in A. japonicus and Aspergillus aculeatus
AUTHOR: Berka R M; Yoder W; Takagi S; Boominathan K C
PATENT ASSIGNEE: Novo-Nordisk-Biotech
PATENT INFO: WO 9515391 8 Jun 1995
APPLICATION INFO: WO 1994-US13613 29 Nov 1994
PRIORITY INFO: US 1993-161675 1 Dec 1993
DOCUMENT TYPE: Patent
LANGUAGE: English

OTHER SOURCE: WPI: 1995-215271 [28]
AN 1995-10491 BIOTECHDS
AB The following are claimed: (1) an Aspergillus japonicus-type host cell including a DNA sequence (I) encoding a heterologous enzyme (II); (2) A. japonicus cells containing a recombinant DNA sequence encoding a homologous enzyme operably linked to a promoter; and (3) a method for the production of recombinant (II) by culturing transformed A. japonicus cells. Preferred cells are A. japonicus, Aspergillus aculeatus or Aspergillus japonicus var. aculeatus. The fungal promoter is Aspergillus oryza TAKA-amylase, **Rhizomucor** miehei aspartic protease or lipase (EC-3.1.1.3), Aspergillus niger glucoamylase (GA, EC-3.2.1.3), neutral or acid-stable **alpha-amylase** (EC-3.2.1.1). (II) includes a catalase (EC-1.11.1.6), laccase (EC-1.10.3.2), phenol-oxidase, oxidoreductase, peroxidase (EC-1.11.1.7), esterase (EC-3.1.1.1), cutinase, aminopeptidase (EC-3.4.11.11), carboxypeptidase, phytase, polygalacturonase (EC-3.2.1.15), pectin-lyase (EC-4.2.2.10), alpha-galactosidase (EC-3.2.1.22), beta-galactosidase (EC-3.2.1.22), mannosidase, beta-D-fructofuranosidase (EC-3.2.1.26), and chitinase (EC-3.2.1.14). (I) is especially fungal lipase, xylanase or cellulase (EC-3.2.1.4). (50pp)

L3 ANSWER 24 OF 26 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1995-10490 BIOTECHDS
TITLE: Aspergillus foetidus cells expressing heterologous enzyme; fungus recombinant lipase, endo-1,4-beta-D-xylanase and cellulase production
AUTHOR: Berka R M; Yoder W; Takagi S; Boominathan K C
PATENT ASSIGNEE: Novo-Nordisk-Biotech
PATENT INFO: WO 9515390 8 Jun 1995
APPLICATION INFO: WO 1994-US13612 29 Nov 1994
PRIORITY INFO: US 1993-160591 1 Dec 1993
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1995-215270 [28]
AN 1995-10490 BIOTECHDS
AB The following are claimed: (1) an Aspergillus foetidus host cell including a DNA sequence (I) encoding a heterologous enzyme (II); (2) A. foetidus cells containing a recombinant DNA sequence encoding a homologous enzyme operably linked to a promoter; and (3) a method for the production of recombinant (II) by culturing transformed A. foetidus cells. The fungal promoter is Aspergillus oryza TAKA-amylase, **Rhizomucor** miehei aspartic protease or lipase (EC-3.1.1.3), Aspergillus niger glucoamylase (GA, EC-3.2.1.3), neutral or acid-stable **alpha-amylase** (EC-3.2.1.1). (II) includes a catalase (EC-1.11.1.6), laccase (EC-1.10.3.2), phenol-oxidase, oxidoreductase, peroxidase (EC-1.11.1.7), esterase (EC-3.1.1.1), cutinase, protease, aminopeptidase (EC-3.4.11.11), carboxypeptidase, phytase, polygalacturonase (EC-3.2.1.15), pectin-lyase (EC-4.2.2.10), GA, alpha-galactosidase (EC-3.2.1.22), beta-galactosidase (EC-3.2.1.22), mannosidase, isomerase, beta-D-fructofuranosidase (EC-3.2.1.26), deoxyribonuclease, or chitinase (EC-3.2.1.14). (I) is especially a fungal enzyme such as lipase, xylanase or cellulase (EC-3.2.1.4). (47pp)

L3 ANSWER 25 OF 26 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:52023 SCISEARCH
THE GENUINE ARTICLE: 998YU
TITLE: Production of saccharogenic and dextrinogenic amylases by **Rhizomucor** pusillus A 13.36
AUTHOR: Silva T M; Attili-Angelis D; Carvalho A F A; Da Silva R; Boscolo M; Gomes E (Reprint)
CORPORATE SOURCE: Univ Estadual Paulista, Lab Bioquim & Microbiol, IBILCE, Sao Jose Do Rio Preto, SP, Brazil (Reprint); UNESP, Inst Biociencias, Dept Bioquim & Microbiol, Rio Claro, SP, Brazil; Univ Estadual Paulista, Lab Fis Quim, IBILCE, Sao Jose Do Rio Preto, SP, Brazil
eleni@bio.ibilce.unesp.br
COUNTRY OF AUTHOR: Brazil
SOURCE: JOURNAL OF MICROBIOLOGY, (DEC 2005) Vol. 43, No. 6, pp. 561-568.

ISSN: 1225-8873.
PUBLISHER: MICROBIOLOGY SOC KOREA, KOREA SCIENCE & TECHNOLOGY CENTER 803, 635-4 YEOGSAM-DONG, KANGNAM-KU, SEOUL 135-703, SOUTH KOREA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 44
ENTRY DATE: Entered STN: 19 Jan 2006
Last Updated on STN: 19 Jan 2006
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A newly-isolated thermophilic strain of the zygomycete fungus Rhizoinucor pusillus 13.36 produced highly active dextrinogenic and saccharogenic enzymes. Cassava pulp was a good alternative substrate for amylase production. Dextrinogenic and saccharogenic amylases exhibited optimum activities at a pH of 4.0-4.5 and 5.0 respectively and at a temperature of 75 degrees C. The enzymes were highly thermostable, with no detectable loss of saccharogenic or dextrinogenic activity, after 1 h and 6 h at 60 degrees C, respectively. The saccharogenic activity was inhibited by Ca2+ while the dextrinogenic was indifferent to this ion. Both activities were inhibited by Fe2+ and Cu2+ Hydrolysis of soluble starch by the crude enzyme yielded 66% glucose, 19.5% maltose, 7.7% maltotriose and 6.6% oligosaccharides.

L3 ANSWER 26 OF 26 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:372461 SCISEARCH
THE GENUINE ARTICLE: 427CF
TITLE: Determination of the disulfide structure of sillucin, a highly knotted, cysteine-rich peptide, by cyanylation/cleavage mass mapping
AUTHOR: Qi J F; Wu J; Somkuti G A; Watson J T (Reprint)
CORPORATE SOURCE: Michigan State Univ, Dept Biochem, E Lansing, MI 48824 USA (Reprint); Michigan State Univ, Dept Chem, E Lansing, MI 48824 USA; USDA, Eastern Reg Res Ctr, Wyndmoor, PA 19038 USA
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (17 APR 2001) Vol. 40, No. 15, pp. 4531-4538
ISSN: 0006-2960.
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 22
ENTRY DATE: Entered STN: 18 May 2001
Last Updated on STN: 18 May 2001
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The disulfide structure of sillucin, a highly knotted, cysteine-rich, antimicrobial peptide, isolated from **Rhizomucor** pusillus, has been determined to be Cys2-Cys7, Cys12-Cys24, Cys13-Cys30, and Cys14-Cys21 by disulfide mass mapping based on partial reduction and CN-induced cleavage enabled by cyanylation. The denatured 30-residue peptide was subjected to partial reduction by tris(2-carboxyethyl)phosphine hydrochloride at pH 3 to produce a mixture of partially reduced sillucin species; the nascent sulfhydryl groups were immediately cyanylated by 1-cyano-4-(dimethylamino)pyridinium tetrafluoroborate. The cyanylated species, separated and collected during reversed phase high-performance liquid chromatography, were treated with aqueous ammonia, which cleaved the peptide chain on the N-terminal side of cyanylated cysteine residues. The CN-induced cleavage mixture was analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry before and after complete reduction of residual disulfide bonds in partially reduced and cyanylated species to mass map the truncated peptides to the sequence. Because the masses of the CN-induced cleavage fragments of both singly and doubly reduced and cyanylated sillucin are related to the linkages of the disulfide bonds in the original molecule, the presence of certain truncated peptide(s) can be used to positively identify the linkage of a specific disulfide bond or exclude the presence of other possible linkages.

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